modification and rescue might involve multiple *Wolbachia* genes.

The existence of *Wolbachia* strains that do not modify sperm but can rescue the cytoplasmic incompatibility phenotype has been predicted by theory[13,14]. Our work suggests that previously classified mod$^-$resc$^-$ strains of *Wolbachia* can in fact rescue cytoplasmic incompatibility and can therefore be considered mod$^-$resc$^+$. This finding helps explain how *Wolbachia* mod$^-$ strains could have spread into insect populations without the action of cytoplasmic incompatibility. *Wolbachia* strains that can rescue but not cause cytoplasmic incompatibility can be expected to increase in frequency in populations by essentially parasitizing the cytoplasmic incompatibility sperm modification effect of a related *Wolbachia* strain.

The potentially lower fitness costs and perfect maternal transmission of these strains[7,9] might explain how they could displace the initial strain that they parasitized in order to invade the host population. This is in accordance with models predictng that *Wolbachia* will evolve towards neutrality with respect to cytoplasmic incompatibility as long as maternal transmission frequency can be increased[13].

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...and discovered on Mount Kilimanjaro

The endocytoplasmic bacterium *Wolbachia* causes the death of arthropod embryos, when present in reproductive organs, by cytoplasmic incompatibility[1]. *Wolbachia* is harboured in both sexes but transmission is maternal only. Cytoplasmic incompatibility occurs when infected males inseminate uninfected females[2] or females bearing a different variant of *Wolbachia*[3,4]. Two kinds of *Wolbachia* have been described[1]: (mod$^+$resc$^+$) which induces cytoplasmic incompatibility by modifying sperm but can rescue this phenotype when in the egg, and (mod$^-$resc$^-$) which neither induces cytoplasmic incompatibility nor rescues from it. Theory predicts a third kind (mod$^-$resc$^+$) that would not induce cytoplasmic incompatibility but would rescue from it[5–7]. We have found such a *Wolbachia* variant in a *Drosophila simulans* population on Mt Kilimanjaro, Tanzania. The existence of a mod$^-$resc$^+$ *Wolbachia* shows that the modification and rescue functions can be dissociated with regard to the cytoplasmic incompatibility process.

We detected this *Wolbachia* variant (*w*Ki) in isofemale lines from flies captured in March 1996. Of 49 lines, 9 showed a positive PCR signal with primers for the *Wolbachia* 16S rRNA gene[8]. Infected (Kili$^+$) and uninfected (Kili$^-$) Kilimanjaro males were crossed with Kili$^-$ females. No cytoplasmic incompatibility was detected. The percentage of unhatched eggs did not differ significantly whether the males were infected or uninfected (12.3 ± 2.6% and 10.7 ± 3.3%, respectively).

We tested the compatibility of Kili$^+$ flies with individuals infected by each of the three natural mod$^+$resc$^+$ *Wolbachia* strains (*w*Ri, *w*Ha or *w*No)[9]. Kili$^+$ males were compatible with females infected by *w*Ri (12.0 ± 5.1% unhatched eggs), *w*Ha (14.0 ± 4.1%) or *w*No (8.5 ± 1.3%). Kili$^+$ females were incompatible with males infected by *w*Ri (100.0% unhatched eggs) or *w*Ha (84.7 ± 3.3%). Surprisingly, the Kili$^+$ females were completely compatible with *w*No-infected males (8.5 ± 2.1% unhatched eggs), whereas Kili$^-$ females were incompatible (70.2 ± 3.5%). *w*Ki thus behaves as a mod$^-$resc$^+$ variant with regard to the *w*No type.

Cytoplasmic incompatibility helps *Wolbachia* in invading previously uninfected populations, because it eliminates uninfected eggs, giving a higher fitness to infected females. However, during the invasion process, any mod$^-$ variant arising by mutation will also increase in frequency as long as it is still resc$^+$. Under certain conditions[7], these mod$^-$resc$^+$ variants would completely replace the mod$^+$resc$^+$ wild type. If this happened, the population would again become vulnerable to invasion by uninfected cytoplasm, because mod$^-$ mutants do not exert any selective pressure against uninfected eggs. The infection might then be lost with time. Meanwhile, because mod$^+$ variants have been eliminated from the population, the rescue capability becomes useless and resc$^-$ mutants can be expected to arise.

The *Wolbachia* variants discovered in wild *D. simulans* populations[9,10] have been of two types: mod$^+$resc$^+$ (*w*Ri, *w*Ha, *w*No) and mod$^-$resc$^-$ (*w*Ma, *w*A). *w*A has been found both on the American and Australian continents, whereas *w*Ma is known only in Madagascar. We established that *w*A and *w*Ki are different because *w*A cannot rescue from the *w*No cytoplasmic incompatibility phenotype[10]. The status of *w*Ma is unknown in this respect, so *w*Ma and *w*Ki might be the same variant. Moreover, the 16S rDNA amplification product of *w*Ki has one *Vsp*I restriction site, as do *w*Ma and *w*No sequences[8], but such a site is absent from *w*Ri, *w*Ha and *w*A sequences.

Eastern Africa is thought to be the centre of origin of *D. simulans*[11], so the *Wolbachia* infection of the Kilimanjaro population could be one of the oldest in this species, implying that mod$^-$resc$^+$ variants derive from a mod$^+$resc$^+$ ancestor with time.

In contrast, *w*No is known only in the Seychelles and in New Caledonia[10], so perhaps *w*No-infected populations originate from migrants that left East Africa bearing the mod$^+$resc$^+$ ancestor. After geographic isolation, the Indo-Pacific variant could have remained mod$^+$resc$^+$ while the ancestral variant has evolved into mod$^-$resc$^+$ in Africa. The maintenance of the rescue function implies that it might have a further role.

The proportion of insect species potentially infected by *Wolbachia* is estimated at 16% (ref. 1), so further research might well yield other mod$^-$resc$^+$ variants. Mt Kilimanjaro is probably just the tip of an iceberg.

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